Litton BIONETICS

MUTAGENIC EVALUATION OF COMPOUND PM 9000593 SHELLAC WAX

(73-51)

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Mutagenic Evaluation of Compound FDA 73-51 **Shellac Wax** 4/15/75

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LBI PROJECT # 2468

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MUTAGENIC EVALUATION OF

COMPOUND PM 9000593

SHELLAC WAX

(73-51)

SUBMITTED TO

FOOD & DRUG ADMINISTRATION
DEPARTMENT OF HEALTH, EDUCATION AND WELFARE
ROCKVILLE, MARYLAND

SUBMITTED BY

LITTON BIONETICS, INC.
5516 NICHOLSON LANE
KENSINGTON, MARYLAND

APRIL 15, 1975

BIONETICS

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EVALUATION SUMMARY

Compound PM9000593, Shellac Wax, was not found to possess significant genetic activity in the series of microbial assays employed in this evaluation.

DATE: 04/15/75

SPONSOR: Food and Drug Administration, Contract Number 223-74-2104

SUBJECT: Evaluation of Test Compound PM 9000593, Shellac Wax

I. OBJECTIVE

The objective of this study was to evaluate the test compound for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations.

II. MATERIALS

A. Test Compound

1. Date Received: August, 1974

2. Description: Hard surface, light brown chunks

B. Indicator Microorganisms

The following strains of indicator microorganisms were used in the evaluation:

Yeast Strain: Saccharomyces cerevisiae, strain D4

Bacteria Strains: Salmonella typhimurium, strains: TA-1535
TA-1537
TA-1538

C. Reaction Mixture

The following reaction mixture was employed in the activation tests:

Component	Final Concentration/ml
1. TPN (sodium salt)	6 μ M
2. Isocitric acid	49 μ M
3. Tris buffer, pH 7.4	28 μ M
4. $MgCl_2$	1.7 μ M
5. Tissue homogenate fraction	72 mg

D. Tissue Homogenates and Supernatant

The tissue homogenates and 9,000 x g supernatants were prepared from tissues of the following mammalian species: Mouse-ICR random bred adult males; rat-sprague-Dawley adult males; and primate-*Macaca mulatta* adult males.

E. Positive Control Compounds

Table 1 lists chemicals for positive controls in the direct and activation assays.

TABLE 1

POSITIVE CONTROLS USED IN DIRECT AND ACTIVATION ASSAYS

Assay	Chemical[a]	Solvent	Probable Mutagenic Specificity[b]
Non-activation	Ethylmethane sulfonate	Water or saline	BPS
	2-Nitrofluorene	Dimethylsulfoxide[c]	FS
	Quinacrine mustard	Water or saline	FS
Activation	Dimethylnitrosamine	Water or saline	BPS
	2-Acetylaminofluorene	Dimethylsulfoxide[c]	FS

[a] Concentrations given in the Results Section
[b] BPS = base-pair substitution; FS = frameshift
[c] Previously shown to be non-mutagenic

III. METHODS

A. Toxicity

The solubility, toxicity and doses for all chemicals were determined prior to screening.

Each chemical was tested for survival against the specific indicator strains over a range of doses to determine the 50% survival dose. Bacteria were tested in phosphate buffer, pH 7.4, for one hour at 37°C on a shaker. Yeasts were tested in phosphate buffer, pH 7.4, for four hours at 30°C on a shaker. The 50% survival curve and the 1/4 and 1/2 50% doses calculated.

If no toxicity was obtained for a chemical with a given strain, then a maximum dose of 5% (w/v) was used against the strain.

Unless otherwise specified, the doses calculated for the tests in buffer were applied to the activation tests. The solubility of the test chemical under treatment conditions is stated in the Results Section.

B. Plate Tests

In the nonactivation procedure, approximately 10^9 cells of a log-phase culture of the bacterial indicator strains were spread over the surface of a minimal plate, and a measured amount of the test chemical was placed in the center of the test plate. In activation tests, the test chemical was added to the cells, and an aliquot of the mixture was spread on the surface of the test plate. The reaction mixture (0.1 ml) plus tissue extract was then spotted on the surface of the plate. Positive and solvent controls were included. All plates were incubated at 37°C for four days and then scored. Each compound (test, positive control and solvent control) was done in duplicate. Concentrations of the positive control compounds are listed in the Results Section.

C. Suspension Tests

1. Non activation

Log-phase bacteria and stationary-phase yeast cultures of the indicator organisms were grown in complete broth, washed and resuspended in 0.9% saline to densities of 1×10^9 cells/ml and 5×10^7 cells/ml, respectively. This constituted the working stock for tests of a group of test chemicals and their respective controls. Tests were conducted in plastic tissue culture plates. Cells plus appropriate volume(s) of the test chemical were added to the wells to give a final volume of 1.5 ml. The solvent replaced the test chemical in the negative controls. Treatment was at 30°C for four hours for yeast tests and at 37°C for one hour for bacterial tests. All flasks were shaken during treatment. Following treatment, the plates were set on ice. Aliquots of cells were removed, diluted in sterile saline (4°C) and plated on the appropriate complete media. Undiluted samples from flasks containing the bacteria were plated on minimal selective medium in reversion experiments. Samples from a 10^{-1} dilution of treated cells were plated on the selected media for enumeration of gene conversion with strain D4. Bacterial plates were scored after incubation for 48 hours at 37°C. The yeast plates were incubated at 30°C for 3-5 days before scoring.

2. Activation

Bacteria and yeast cells were grown and prepared as described in the non activation tests. Measured amounts of the test and control chemicals plus 0.25 ml of the stock-cell suspension were added to wells of the Linbro plate containing the appropriate tissue fraction and reaction mixture. All flasks (bacteria and yeast) were incubated at 37°C in an oxygen atmosphere with shaking. The treatment times as well as the dilutions, plating procedures and scoring of the plates were the same as described for non activation tests.

D. Preparation of Tissue Homogenates and 9,000 x g Cell Fractions

Male animals (sufficient to provide the necessary quantities tissues) were killed by cranial blow, decapitated and bled. Organs were immediately dissected from the animal using aseptic techniques and placed in ice-cold 0.25 M sucrose buffered with Tris at pH of 7.4. Upon collection of the desired quantity of organs, they were washed twice with fresh buffered sucrose and completely homogenized with a motor-driven homogenizing unit at 4°C. The whole organ homogenate obtained from this step was divided into two samples. One sample was frozen at -80°C and the other was centrifuged for 20 minutes at 9,000 x g in a refrigerated centrifuge. The supernatant from the centrifuged sample was retained and frozen at -80°C. These two frozen samples were used for the activation studies.

E. Data Recording and Reporting

Following the specified incubation periods all population plates were scored by an automatic colony counter and the results from each plate of a set were recorded, in ink, on data processing forms. All minimal or other types of selective media plates were hand scored and the results recorded along with the respective population data. Other relevant experimental data were recorded on experimental definition forms. For bacteria strains the number of colonies recorded from either the population or selective plates represents that number in 1 ml of test suspension plated. The numbers recorded for the yeast strain D4 represent the number in 0.5 ml of test suspension plated. Data was then processed and printed from a computer program.

TOXICITY AND DOSAGE DETERMINATIONS

COMPOUND PM 9000593 TEST DATE: January 7, 1975

Range of concentrations of the test compound used to determine the 50% survival level

Dose Number	% Concentration
1	10.0
2	1.0
3	0.1
4	0.01
5	0.001

Concentrations of the test chemical required for mutagenicity tests

Dose	% Concentration	
	Bacteria	Yeast
1/4 50% survival	0.015	0.25
1/2 50% survival	0.030	0.50
50% survival	0.060	1.00
Plate Test	0.030	--

SOLUBILITY PROPERTIES OF THE TEST COMPOUND

1. NAME OR DESCRIPTION OF TEST COMPOUND:

   Shellac Wax PM 9000593

2. TEST SOLVENT AND DESCRIPTION OF SOLUBILITY:

   Suspension in 10% DMSO Not soluble under treatment conditions.

3. OTHER COMMENTS:

   Light brown chunks

C. Summary of Test Results

Plate Tests

1. Name or code designation of the test compound: PM 9000593

2. Test date: January 31, 1975

3. Concentration of the test compound: 0.03%

Test	Species	Tissue	TA-1535		TA-1537		TA-1538	
Non-activation			1	2	1	2	1	2
Solvent Control	--	--	6	1	4	3	6	2
Positive Control[a]	--	--	$>10^4$	$>10^4$	84	74	34	40
Test Compound	--	--	7	4	3	0	3	3
Activation								
Negative Control	--	--	11	12	1	1	12	9
Solvent Control	--	--	4	9	3	1	6	5
Reaction Mixture Control	--	--						
Positive Control[b]	Mouse	Liver	>500	>500	>100	85	>200	>200
Positive Control		Lung	9	5	8	11	15	13
Positive Control		Testes	3	4	7	8	10	9
Positive Control	Rat	Liver	>100	>100	28	24	63	63
Positive Control		Lung	9	4	6	7	12	8
Positive Control		Testes	4	3	8	6	9	12
Positive Control	Monkey	Liver	>100	>100	38	25	31	28
Positive Control		Lung	10	5	6	7	12	6
Positive Control		Testes	4	5	6	6	10	10
Test Compound	Mouse	Liver	7	5	6	6	6	6
Test Compound		Lung	3	7	2	2	4	9
Test Compound		Testes	6	6	2	3	5	7
Test Compound	Rat	Liver	7	6	5	6	8	8
Test Compound		Lung	3	7	1	2	3	9
Test Compound		Testes	4	6	4	4	5	10
Test Compound	Monkey	Liver	5	5	3	8	7	9
Test Compound		Lung	4	9	1	2	3	9
Test Compound		Testes	5	6	3	4	5	6

[a]
TA-1535	EMs	10 µl/plate
TA-1537	QM	20 µg/plate
TA-1538	NF	100 µg/plate

[b]
TA-1535	DMNA	50 µm/plate
TA-1537	AAF	100 µg/plate
TA-1538	AAF	100 µg/plate

DATA TABLE TERMS AND ABBREVIATIONS

ABBREVIATION OR TERM	DEFINITION OR EXPLANATION
COMPOUND	Client designated compound number appears in this column.
TEST CODES	NAN = Non Activation: Solvent Control NAP = Non Activation: Positive Control NA1 = Non Activation: Test Compound Dose 1 NA2, etc. = Reflects the other dose level(s) A+C = Negative Chemical Control A-C = Activation: Solvent Control ACP = Activation: Positive Control ACT = Activation: Test Compound LI = Liver Tissue Activation Fraction LU = Lung Tissue Activation Fraction KI = Kidney Tissue Activation Fraction TE = Testes Tissue Activation Fraction 1,2, etc. = Dose Levels
CONCENTRATION	All test compound dose levels are expressed as a whole number followed by an exponent (negative) identified by the appropriate units. Example: 0025-2PCT = 0.25 percent concentration
POPU	Total number of viable cells in the plating sample raised to some exponent printed directly below the abbreviation (i.e., EP + 6 = X 10^6).
MUT 1	Total number of mutants or convertants obtained from the sample plated raised to some exponent printed directly below the abbreviation (i.e., EP + 0 = X 10^0). For strain D4, MUT 1 represents the number of ADE+ convertants.
MUT 2	Only used for strain D4 and represents the number of TRY+ convertants in the plated sample.
FREQ 1	The calculated mutation or gene conversion frequency times the negative exponent written directly below. For strain D4, FREQ 1 represents the ADE+ value.
FREQ 2	Only used for strain D4 and represents the TRY+ conversion frequency.
CONTAM	Presence of contamination on any plates.

DATA TABLE TERMS AND ABBREVIATIONS (continued)

ABBREVIATION OR TERM	DEFINITION OR EXPLANATION
AAF	2-Acetylaminofluorene
DMSO	Dimethylsulfoxide
DMN	Dimethylnitrosamine
EMS	Ethyl Methanesulfonate
QM	Quinacrine Mustard
NF	Nitrofluorene
SPECIES	Animal Strains
SPRDAW	Sprague Dawley Rats
ICRFLO	Flow ICR Random Bred Mice
RHESUS	Rhesus Monkey (Macaca mulatta)
MIXEDB	Dog, Mixed Breed
NEWZEA	New Zealand White Rabbit

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 04/15/75

SPECIES COMPOUND PM9000593

TEST	ORG	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	000004 ADE EX-5	000004 TRY EX-5
NAN		1.61	12.08	5.80	2.21	3.14
NAP		243.05	2686.46	469.44	66.33	77.39
NA1		1.89	17.30	12.56	1.73	3.96
NA2		1.68	5.90	7.20	2.44	2.79

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 04/15/75

SPECIES ICRFLO COMPOUND PM9000593

TEST	ORG	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	000004 ADE EX-5	000004 TRY EX-5
ACT	A+C	0.93	3.59	4.92	6.30	12.91
ACT	A-C	1.04	3.21	5.77	6.38	13.81
ACT	PLI	128.68	6.71	24.04	9.48	29.12
ACT	PLU	1.00	2.19	7.37	6.67	16.81
ACT	PTE	1.63	2.90	7.02	4.73	17.40
ACT	LI1	2.84	2.51	9.66	4.89	18.74
ACT	LI2	2.49	1.98	7.70	3.96	16.61
ACT	LU1	1.56	2.13	5.55	5.50	18.50
ACT	LU2	2.23	2.92	8.84	4.39	17.40
ACT	TE1	1.45	3.11	7.39	4.26	19.07
ACT	TE2	2.31	2.90	9.40	4.21	16.67

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 04/15/75

SPECIES SPRDAW COMPOUND PM9000593

TEST	ORG	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	000004 ADE EX-5	000004 TRY EX-5
ACT	A+C	2.22	6.77	7.88	5.52	14.73
ACT	A-C	1.45	2.70	6.66	7.34	18.04
ACT	PLI	184.24	44.71	27.00	9.87	21.94
ACT	PLU	2.45	4.96	10.52	6.34	16.64
ACT	PTF	2.83	4.50	8.02	1.63	2.90
ACT	LI1	2.19	20.45	12.23	5.04	23.26
ACT	LI2	2.77	15.96	12.63	3.39	14.83
ACT	LU1	0.96	6.56	7.40	2.54	19.78
ACT	LU2	0.84	3.39	12.90	5.39	21.12
ACT	TF1	3.53	4.90	8.38	3.42	14.80
ACT	TF2	2.58	4.73	8.97	3.64	13.81

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 04/15/75

SPECIES RHESUS COMPOUND PM9000593

TEST	ORG	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	0000D4 ADE EX-5	0000D4 TRY EX-5
ACT	A+C	6.27	11.64	8.12	3.21	49.15
ACT	A-C	3.13	0.52	6.60	5.18	45.95
ACT	PLI	52.59	9.57	24.07	6.74	79.77
ACT	PLU	5.62	5.13	10.43	1.01	68.34
ACT	PTE	6.42	8.91	6.49	3.76	42.38
ACT	LI1	3.65	1.87	6.91	4.76	30.30
ACT	LI2	3.94	4.08	10.94	5.38	65.47
ACT	LU1	4.13	2.16	14.98	2.47	41.37
ACT	LU2	3.76	5.52	11.51	5.74	58.11
ACT	TE1	3.24	2.24	11.89	4.39	66.55
ACT	TE2	4.74	2.63	12.32	2.70	45.45

V. INTERPRETATION OF RESULTS AND CONCLUSIONS

Compound PM9000593, Shellac Wax, was evaluated for genetic activity in a series of *in vitro* microbial assays with and without metabolic activation. The following results were obtained:

A. *Salmonella typhimurium*

1. Plate tests

At a concentration of 0.03%, this chemical was not mutagenic for TA-1535, TA-1537 or TA-1538 in either direct or activation assays.

2. Nonactivation suspension tests

The results of these tests were negative. A slight increase was noted for the NA1 with TA-1538, but it was not considered significant.

3. Activation suspension tests

The results of these tests were negative. It was noted that the positive control values for TA-1537 and TA-1538 were lower than expected in all cases.

B. *Saccharomyces cerevisiae*

1. Nonactivation suspension tests

The results of these tests were negative.

2. Activation suspension tests

The results of these tests were negative. The positive control results appeared low for the TRY locus but the difference was likely due to a higher than normal background frequency of convertants.

C. Conclusions

Compound PM9000593 was not found to possess any significant genetic activity based on the results of the microbial assays employed in this evaluation.

Submitted by:

David Brusick, Ph.D.
Director of Genetics

APPENDIX

Tabulation of Data

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 500902 DETECTOR TA1535 SPECIES DATE - 04/15/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-8	CONTAM
	NAN		SALINE	1308	0021	1.61	0
	NAP		EMS 0.002 %	1057	2569	243.05	0
PM9000593	NA1		0003-2 PCT.	0795	0015	1.89	0
PM9000593	NA2		0015-3 PCT.	1247	0021	1.68	2

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 502302 DETECTOR TA1537 SPECIES DATE - 04/15/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	NAN		SALINE	0240	0029	12.08	0
	NAP		0M 1.0 UG/ML	0096	2579	**2686.46**	0
PM9000593	NA1		0003-2 PCT.	0370	0064	17.30	0
PM9000593	NA2		0015-3 PCT.	0525	0031	5.90	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 502301 DETECTOR TA1538 SPECIES DATE - 04/15/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	NAN		DMSO	0414	0024	5.80	0
	NAP		NF 125 UG-ML	0288	1352	469.44	0
PM9000593	NA1		0003-2 PCT.	0199	0025	12.56	0
PM9000593	NA2		0015-3 PCT.	0250	0018	7.20	2

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 504801 DETECTOR 000004 SPECIES DATE - 04/15/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	NAN		SALINE	1084	0024	0034	2.21	3.14	0
	NAP		EMS 1.0 %	1004	0666	0777	66.33	77.39	0
PM9000593	NA1		0005-1 PCT.	0808	0014	0032	1.73	3.96	0
PM9000593	NA2		0025-2 PCT.	0861	0021	0024	2.44	2.79	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 434601 DETECTOR TA1535 SPECIES ICRFLO DATE - 04/15/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		DMN 50 UM/ML	1286	0012	0.93	0
	A-C		SALINE	1248	0013	1.04	2
	ACP	LI	DMN 50 UM/ML	1210	1557	128.68	0
	ACP	LU	DMN 50 UM/ML	1095	0011	1.00	2
	ACP	TE	DMN 50 UM/ML	1163	0019	1.63	2
PM9000593	ACT	LI1	0003-2 PCT.	1128	0032	2.84	2
PM9000593	ACT	LI2	0015-3 PCT.	1003	0025	2.49	2
PM9000593	ACT	LU1	0003-2 PCT.	1155	0018	1.56	0
PM9000593	ACT	LU2	0015-3 PCT.	1123	0025	2.23	2
PM9000593	ACT	TE1	0003-2 PCT.	1103	0016	1.45	2
PM9000593	ACT	TE2	0015-3 PCT.	1167	0027	2.31	2

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM

COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468

EXPERIMENT 434701 DETECTOR TA1537 SPECIES ICRFLO DATE - 04/15/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-8	CONTAM
	A+C		AAF 800 UG/ML	1783	0064	3.59	0
	A-C		DMSO	1590	0051	3.21	0
	ACP	LI	AAF 800 UG/ML	1983	0133	6.71	3
	ACP	LU	AAF 800 UG/ML	1460	0032	2.19	2
	ACP	TE	AAF 800 UG/ML	1619	0047	2.90	2
PM9000593	ACT	LI1	0003-2 PCT.	1794	0045	2.51	2
PM9000593	ACT	LI2	0015-3 PCT.	1617	0032	1.98	2
PM9000593	ACT	LU1	0003-2 PCT.	1595	0034	2.13	2
PM9000593	ACT	LU2	0015-3 PCT.	1303	0038	2.92	0
PM9000593	ACT	TE1	0003-2 PCT.	1608	0050	3.11	2
PM9000593	ACT	TE2	0015-3 PCT.	1967	0057	2.90	2

REPORT FXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM

COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468

EXPERIMENT 435001 DETECTOR TA1538 SPECIES ICRFLO DATE - 04/15/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		AAF 800 UG/ML	1363	0067	4.92	0
	A-C		DMSO	1820	0105	5.77	0
	ACP	LI	AAF 800 UG/ML	1252	0301	24.04	3
	ACP	LU	AAF 800 UG/ML	1262	0093	7.37	2
	ACP	TE	AAF 800 UG/ML	1140	0080	7.02	2
PM9000593	ACT	LI1	0003-2 PCT.	0932	0090	9.66	2
PM9000593	ACT	LI2	0015-3 PCT.	1546	0119	7.70	2
PM9000593	ACT	LU1	0003-2 PCT.	1675	0093	5.55	0
PM9000593	ACT	LU2	0015-3 PCT.	1278	0113	8.84	2
PM9000593	ACT	TE1	0003-2 PCT.	1461	0108	7.39	2
PM9000593	ACT	TE2	0015-3 PCT.	1074	0101	9.40	2

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 500701 DETECTOR 00004 SPECIES ICRFLD DATE - 04/15/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	A+C		DMN 90 UM/ML	0968	0061	0125	6.30	12.91	0
	A-C		SALINE	1050	0067	0145	6.38	13.81	0
	ACP	LI	DMN 90 UM/ML	0728	0069	0212	9.48	29.12	?
	ACP	LU	DMN 90 UM/ML	0809	0054	0136	6.67	16.81	?
	ACP	TE	DMN 90 UM/ML	0931	0044	0162	4.73	17.40	6
PM9000593	ACT	LI1	0005-1 PCT.	0491	0024	0092	4.89	18.74	0
PM9000593	ACT	LI2	0025-2 PCT.	0632	0025	0105	3.96	16.61	0
PM9000593	ACT	LU1	0005-1 PCT.	0400	0022	0074	5.50	18.50	?
PM9000593	ACT	LU2	0025-2 PCT.	0638	0028	0111	4.39	17.40	1
PM9000593	ACT	TF1	0005-1 PCT.	0493	0021	0094	4.26	19.07	0
PM9000593	ACT	TF2	0025-2 PCT.	0618	0026	0103	4.21	16.67	2

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 500201 DETECTOR TA1535 SPECIES SPRDAW DATE - 04/15/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-8	CONTAM
	A+C		DMN 50 UM/ML	0450	0010	2.22	0
	A-C		SALINE	0826	0012	1.45	?
	ACP	LI	DMN 50 UM/ML	0628	1157	184.24	0
	ACP	LU	DMN 50 UM/ML	0653	0016	2.45	0
	ACP	TE	DMN 50 UM/ML	0566	0016	2.83	?
PM9000593	ACT	LI1	0003-2 PCT.	0639	0014	2.19	?
PM9000593	ACT	LI2	0015-3 PCT.	0506	0014	2.77	?
PM9000593	ACT	LU1	0003-2 PCT.	0728	0007	0.96	0
PM9000593	ACT	LU2	0015-3 PCT.	0476	0004	0.84	?
PM9000593	ACT	TE1	0003-2 PCT.	0538	0019	3.53	?
PM9000593	ACT	TE2	0015-3 PCT.	0427	0011	2.58	?

REPEAT

REPORT FXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 502101 DETECTOR TA1537 SPECIES SPRDAW DATE - 04/15/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		AAF 800 UG/ML	0251	0017	6.77	0
	A-C		DMSO	0185	0005	2.70	0
	ACP	LI	AAF 800 UG/ML	0170	0076	44.71	2
	ACP	LU	AAF 800 UG/ML	0141	0007	4.96	0
	ACP	TE	AAF 800 UG/ML	0111	0005	4.50	0
PM9000593	ACT	LI1	0003-2 PCT.	0220	0045	20.45	2
PM9000593	ACT	LI2	0015-3 PCT.	0282	0045	15.96	2
PM9000593	ACT	LU1	0003-2 PCT.	0061	0004	6.56	0
PM9000593	ACT	LU2	0015-3 PCT.	0177	0006	3.39	2
PM9000593	ACT	TE1	0003-2 PCT.	0102	0005	4.90	2
PM9000593	ACT	TE2	0015-3 PCT.	0148	0007	4.73	2

REPEAT TEST

Compound	Test	Population	Mutants	Frequency ($X10^{-8}$)
	A-C DMSO	0601	32	5.32
PM900593	LI 1 0003-2 PCT.	0688	26	3.78
PM900593	LI 2 0015-3 PCT.	0646	33	5.11

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 500301 DETECTOR TA1538 SPECIES SPRDAW DATE - 04/15/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		AAF 800 UG/ML	0964	0076	7.88	0
	A-C		DMSO	1276	0085	6.66	1
	ACP	LI	AAF 800 UG/ML	1052	0284	27.00	0
	ACP	LU	AAF 800 UG/ML	1017	0107	10.52	0
	ACP	TE	AAF 800 UG/ML	1347	0108	8.02	2
PM9000593	ACT	LI1	0003-2 PCT.	0466	0057	12.23	0
PM9000593	ACT	LI2	0015-3 PCT.	0784	0099	12.63	0
PM9000593	ACT	LU1	0003-2 PCT.	0500	0037	7.40	2
PM9000593	ACT	LU2	0015-3 PCT.	0783	0101	12.90	2
PM9000593	ACT	TE1	0003-2 PCT.	0585	0049	8.38	2
PM9000593	ACT	TE2	0015-3 PCT.	0992	0089	8.97	0

REPORT FXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 500801 DETECTOR 000004 SPECIES SPRDAW DATE - 04/15/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	A+C		DMN 90 UM/ML	0706	0039	0104	5.52	14.73	0
	A-C		SALINE	0654	0048	0118	7.34	18.04	0
	ACP	LI	DMN 90 UM/ML	0679	0067	0149	9.87	21.94	0
	ACP	LU	DMN 90 UM/ML	0631	0040	0105	6.34	16.64	0
	ACP	TE	DMN 90 UM/ML	0861	0014	0025	1.63	2.90	0
PM9000593	ACT	LI1	0005-1 PCT.	0516	0026	0120	5.04	23.26	4
PM9000593	ACT	LI2	0025-2 PCT.	0472	0016	0070	3.39	14.83	0
PM9000593	ACT	LU1	0005-1 PCT.	0551	0014	0109	2.54	19.78	4
PM9000593	ACT	LU2	0025-2 PCT.	0464	0025	0098	5.39	21.12	0
PM9000593	ACT	TE1	0005-1 PCT.	0527	0018	0078	3.42	14.80	0
PM9000593	ACT	TE2	0025-2 PCT.	0659	0024	0091	3.64	13.81	4

REPORT FXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM

COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468

EXPERIMENT 500901 DETECTOR TA1535 SPECIES RHESUS DATE - 04/15/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		DMN 50 UM/ML	0734	0046	6.27	0
	A-C		SALINE	1119	0035	3.13	2
	ACP	LI	DMN 50 UM/ML	0945	0497	52.59	3
	ACP	LU	DMN 50 UM/ML	0908	0051	5.62	0
	ACP	TE	DMN 50 UM/ML	0748	0048	6.42	2
PM9000593	ACT	LI1	0003-2 PCT.	0987	0036	3.65	2
PM9000593	ACT	LI2	0015-3 PCT.	0989	0039	3.94	0
PM9000593	ACT	LU1	0003-2 PCT.	1066	0044	4.13	2
PM9000593	ACT	LU2	0015-3 PCT.	0930	0035	3.76	0
PM9000593	ACT	TE1	0003-2 PCT.	1049	0034	3.24	0
PM9000593	ACT	TE2	0015-3 PCT.	0949	0045	4.74	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 502201 DETECTOR TA1537 SPECIES RHESUS DATE - 04/15/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		AAF 800 UG/ML	0146	0017	11.64	0
	A-C		DMSO	0192	0001	0.52	0
	ACP	LI	AAF 800 UG/ML	0188	0018	9.57	0
	ACP	LU	AAF 800 UG/ML	0156	0008	5.13	2
	ACP	TE	AAF 800 UG/ML	0101	0009	8.91	0
PM9000593	ACT	LI1	0003-2 PCT.	0374	0007	1.87	0
PM9000593	ACT	LI2	0015-3 PCT.	0196	0008	4.08	0
PM9000593	ACT	LU1	0003-2 PCT.	0232	0005	2.16	0
PM9000593	ACT	LU2	0015-3 PCT.	0145	0008	5.52	0
PM9000593	ACT	TE1	0003-2 PCT.	0312	0007	2.24	0
PM9000593	ACT	TE2	0015-3 PCT.	0114	0003	2.63	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 501001 DETECTOR TA1538 SPECIES RHESUS DATE - 04/15/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		AAF 800 UG/ML	0936	0076	8.12	0
	A-C		DMSO	1076	0071	6.60	?
	ACP	LI	AAF 800 UG/ML	0810	0195	24.07	3
	ACP	LU	AAF 800 UG/ML	1064	0111	10.43	0
	ACP	TE	AAF 800 UG/ML	1263	0082	6.49	?
PM9000593	ACT	LI1	0003-2 PCT.	0781	0054	6.91	?
PM9000593	ACT	LI2	0015-3 PCT.	0649	0071	10.94	?
PM9000593	ACT	LU1	0003-2 PCT.	0621	0093	14.98	0
PM9000593	ACT	LU2	0015-3 PCT.	0747	0086	11.51	0
PM9000593	ACT	TE1	0003-2 PCT.	0883	0105	11.89	?
PM9000593	ACT	TE2	0015-3 PCT.	0763	0094	12.32	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 502901 DETECTOR 000004 SPECIES RHESUS DATE - 04/15/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	A+C		DMN 90 UM/ML	0529	0017	0260	3.21	49.15	4
	A-C		SALINE	0618	0032	0284	5.18	45.95	0
	ACP	LI	DMN 90 UM/ML	0341	0023	0272	6.74	79.77	0
	ACP	LU	DMN 90 UM/ML	0398	0004	0272	1.01	68.34	2
	ACP	TE	DMN 90 UM/ML	0505	0019	0214	3.76	42.38	0
PM9000593	ACT	LI1	0005-1 PCT.	0462	0022	0140	4.76	30.30	0
PM9000593	ACT	LI2	0025-2 PCT.	0223	0012	0146	5.38	65.47	0
PM9000593	ACT	LU1	0005-1 PCT.	0365	0009	0151	2.47	41.37	2
PM9000593	ACT	LU2	0025-2 PCT.	0296	0017	0172	5.74	58.11	4
PM9000593	ACT	TE1	0005-1 PCT.	0296	0013	0197	4.39	66.55	0
PM9000593	ACT	TE2	0025-2 PCT.	0407	0011	0185	2.70	45.45	0